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Skin antisepsis protocols for the collection of blood from donor dogs

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ABSTRACT: *The objective of this study was to evaluate and compare the bactericidal efficacy of2% chlorhexidine surfactant solution + 70% alcohol and 2% chlorhexidine surfactant solution + 0.5% chlorhexidine-alcohol, and standardize skin antisepsis for blood collection from donor dogs. One hundred and twenty skin swabsof the jugular regions of 20 dogs were evaluated. Swabs were distributed into six treatment(T) groups according to the disinfectant used and removal or retention of local hair: T1involved neither antisepsisnorhair removal; T2comprised 2% chlorhexidine + 0.5% chlorhexidine-alcoholwithout hair removal;T3 comprised 2% chlorhexidine + 70% alcohol without hair removal; T4comprised hair removal but no antisepsis;T5comprised 2% chlorhexidine + 0.5% chlorhexidine-alcohol withhair removal; and T6comprised 2% chlorhexidine + 70% alcohol with hair removal. Antiseptic agents were continuously applied in a single direction for a total of 3 min. Use of antiseptics was effective with or without hair removal, resulting in the absence of bacterial growth. Complete efficacy of the technique used in this study may have been due to the increased antiseptic application time. In conclusion,the antisepsis protocols tested in this study can be safely used for the collection of blood from dogs; although,removal of hair prior to antisepsis is still recommended.*
Key words: *bacterial contamination; blood collection; disinfection, chlorhexidine, dog.*

Protocolos de antissepsia de pele para colheita de sangue de cães doadores

RESUMO: *O objetivo deste estudo foi avaliar e comparar o potencial de redução bacteriana proporcionado peloclorexidinadegermante 2% + álcool 70% eclorexidinadegermante 2% + clorexidinaalcoolica 0,5% e padronizar a antissepsia de pele para colheita de sangue de cães doadores. Foram avaliados 120 zaragatoas de pele da região da jugular de 20 cães, que foram distribuídos em seis tratamentos (T) de acordo com oagente usado para desinfecção, associado, ou não, a tricotomia local: T1 – Tratamento sem tricotomia e sem antissepsia, T2 - Tratamento clorexidinadegermante 2% + clorexidinaalcoolica 0,5% sem tricotomia, T3 – Tratamento clorexidina 2% + álcool 70% sem tricotomia, T4 - Tratamento com tricotomia e sem antissepsia, T5 - Tratamento clorexidina 2% + clorexidina alcoólica 0,5% com tricotomia, T6 - Tratamento clorexidina 2% + álcool 70% com tricotomia. A antissepsia foi feita de forma contínua em um único sentido, totalizando 3 minutos. O uso dos antissépticos se mostraram eficazes nos tratamentos com e sem tricotomia não apresentando crescimento bacteriano. A eficácia de 100% da técnica utilizada no presente trabalho pode ser decorrente do maior tempo de antissepsia. Conclui-se que os protocolos de antissepsia realizados neste estudo podem ser utilizados com segurança para a colheita de sangue de cães, embora ainda o recomendado seja a tricotomia antes da antissepsia.*
Palavras-chave: *contaminação bacteriana; colheita de sangue; desinfecção, clorexidina, cão.*

INTRODUCTION

Bacterial contamination of blood components meant for transfusion is a major concern in hemotherapeutic practice and is currently the main cause of blood transfusion-associated infections(BRECHER & HAY, 2005). Methods to prevent bacterial contamination of collected blood are mainly based on donor cutaneous antisepsis and the deviation of the initial blood flow during collection (PEREZ et al., 2002).

Antisepsis is the prevention of sepsis by the exclusion, destruction, or inhibition of the growth of microorganisms in tissues and body fluids (FOSSUM, 2013). Awareness of the importance of decontaminating living tissues has increased, mainly due to a realization that the patient is the primary source of infection, since microorganisms live on the skin surface, especially on the corneous layer, as well as inside the sweat glands, sebaceous follicles, and hair follicles (RODRIGUES et al., 1997). Knowledge

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of the transmission pathways of infection-causing microorganisms and the identification of the bacteria involved in contamination may reduce the occurrence and severity of such infections (SLATTER, 1993).

The transient microbiota is composed of recent environmental contaminants that survive on the skin for short periods. Resident microorganisms, such as coagulase-negative staphylococci, species of *Corynebacterium*, *Propionibacterium*, and *Acinetobacter*, and certain members of the *Klebsiella-Enterobacter* group, cannot be removed by simple washing. Instead, their removal requires the use of antiseptic solutions with antimicrobial properties. An adequate antiseptic should exert germicidal effects on the mucosal and cutaneous microbiota in the presence of blood, serum, mucus, or pus without irritating the skin or mucous membranes (SILVA et al., 2000).

The three main antiseptic formulations in the market are aqueous, alcoholic, and detergent or surfactant solutions. Aqueous formulations are used primarily for mucosal antisepsis, alcoholic solutions are used for the antisepsis of whole skin, and detergent solutions are used toremove impurities from the skin surface (CELERE, 2011).

Although, donations to dog blood banks and the practice of blood transfusion between dogs have become more frequent, standardized pre-collection procedures are lacking. Storage is the biggest aggravating factor, as microorganisms may proliferate in contaminated blood. Patients requiring blood transfusion are typically in critical conditions, thereby experiencingan increased risk of sepsis and death (PEREIRA & RAMALHO, 2001). Therefore, given that bacteria present in and on the skin at the time of venipuncture represent the greatest source of contamination of stored blood, the identification of effective antisepsis protocols is necessary.

Thus, the aim of this study was to evaluate and compare the bactericidal potential ofcombinations of 2% chlorhexidine surfactant solution + 70% alcohol and 2% chlorhexidine surfactant solution + 0.5% chlorhexidine-alcohol,and to standardize skin antisepsis for blood collection from canine donors.

MATERIALS AND METHODS

Samples were collected from 20 clinically healthy dogs by swabbing their neck sat the two jugular regions, using sterile cotton and washed swabs soaked in 0.5ml sterile saline. Samples constituted six treatment(T) groups, classified according to the disinfectant used and whether or not hair was removed locally:T1 involved neither antisepsis nor hair removal; T2 compriseda combination of 2% chlorhexidine surfactant solution + 0.5% chlorhexidine-alcohol without hair removal; T3 compriseda combination of 2%chlorhexidine surfactant solution + 70% alcohol without hair removal; T4 comprised hair removal but no antisepsis; T5 compriseda combination of 2% chlorhexidine surfactant solution + 0.5% chlorhexidine-alcohol with hair removal; and T6 compriseda combination of 2% chlorhexidine surfactant solution + 70% alcohol with hair removal. In total, 120 swabs were collected. Allantiseptics used in the study are from Riohex®, Bioquímica.

T1 and T4 were performed in the jugular area. T2 and T3 were administered to the cranial and caudal portions, respectively, of the right jugular area. The T5 and T6 were administered to the cranial and caudal portions, respectively, of the left jugular area.

Antisepsis was continuously applied in a single direction with gauze for 1.5min per antiseptic, totaling 3min of treatment. Samples were collected immediately afterwards. The swabs were inoculated intothe brain heart infusion (BHI) broth (Neogen Corporation®) and kept for 24 to 72h in a 002 CB-Fanem LTDA® incubator at 37°C. Bacterial growth was evaluated by observing the turbidity of the BHI broth.

Positive samples were seeded in blood agar (Himedia® nutrient agar and sheep's blood) and Mac Conkey agar (Himedia®) (QUINN et al., 2005) and incubated at 37°C for 24h (OLIVEIRA, 2000). Microorganisms were then identified by Gram staining (OLIVEIRA, 2000).

RESULTS AND DISCUSSION

Antisepsis was effective with or without hair removal. After 24 to 72h of incubation no bacterial growth was observed in the BHI broth inoculated with samples from the groups in which antiseptics were used (Figure 1). However, all samples in the T1 and T4 control groups, which were collected without prior antisepsis, showedbacterial growth. Of the bacteria cultured from the T1 and T4 samples, 85% (17/20) and 75% (15/20), respectively,were gram-positive cocci.

These bacterial isolates were similar to those found by SWAIM et al. (1991) and CELERE (2011), who noted thatgram-positive bacteria predominate in the microbiota of canines and humans. Individual characteristics of each animal and the environments in which they live contribute to variations in the cutaneous microbial load and the microorganism and species present (GRICE & SEGRE, 2011).

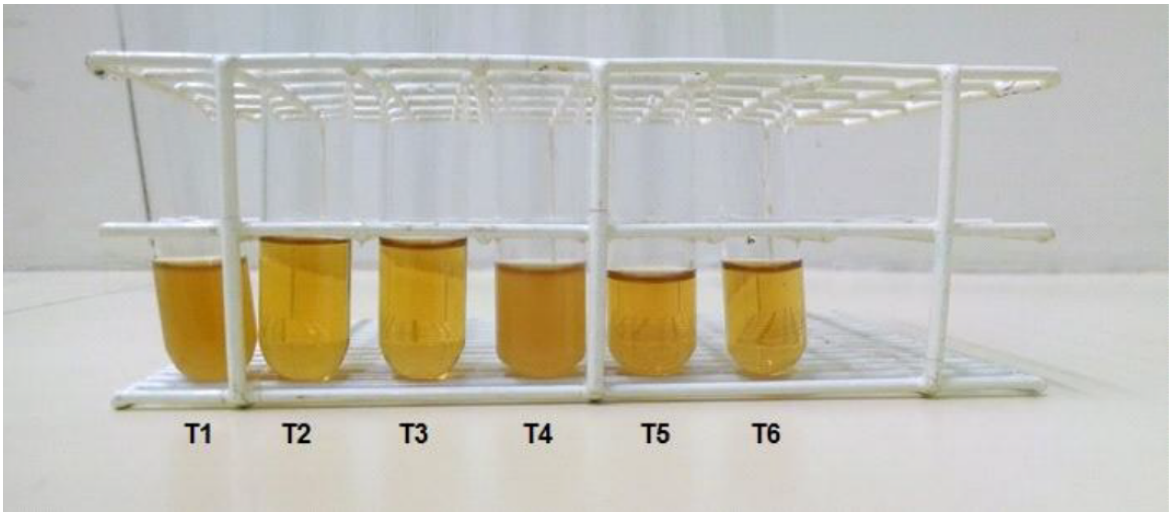

Figure 1 - Comparison of bacterial growth between the T1-T6 groups after culture in BHI liquid with a 24-hour incubation period. Turbidity of the T1 and T4 groups are compatible with bacterial growth, while the BHI liquid remained clear in the other groups, indicating the absence of bacterial growth.

T1 involved neither antisepsis nor hair removal; T2 comprised a combination of 2% chlorhexidine surfactant solution + 0.5% chlorhexidine-alcoholwithout hair removal; T3 comprised a combination of 2%chlorhexidine surfactant solution + 70% alcohol without hair removal; T4 comprised hair removal but no antisepsis; T5 comprised a combination of 2% chlorhexidine surfactant solution + 0.5% chlorhexidine-alcohol with hair removal; and T6 comprised a combination of 2% chlorhexidine surfactant solution + 70% alcohol with hair removal.Source: author.

In this study, the antisepsis protocols used resulted in a 100% reduction in the number of bacteria present on canine skin even without hair removal. However, according to PAVLETIC (2010), animal hair acts as a physical barrier that can retain dirt and microorganisms, making antisepsis more difficult. Removal of hair is thus an important procedure that aims to reduce the risk of contamination during blood collection from canine donors, increasing transfusion safety.

ARCOS & GOLDMAN (2010) evaluated the effectiveness of applying 2% chlorhexidine gluconate + 70% alcohol and 2% iodine tincture + 70% alcohol antisepsis protocols to humans. These authors recorded a 99% reduction in bacteria, and noted that more significant results were obtained using the former combination.

According to ALTEMEIER (1991), alcohol at an appropriate concentration is an efficient and effective antiseptic that reduces the number of microorganisms on the skin by denaturing microbial proteins and interfering with microbial metabolism (OLIVEIRA, 2005). MORIYA &MÓDENA (2008) reported that ethyl and isopropyl alcohols at concentrations of 70% and 92%, respectively, exert almost immediate germicidal effects. However, these treatments had no residual activity, and their repeated application resulted in dry skin.

Solutions containing chlorhexidine are highly antimicrobial, acting approximately 15 s after application against a wide spectrum of gram-positive and gram-negative bacteria. In addition, the toxicity of chlorhexidine is low (DENTON, 2001). In this study, 70% alcohol and 2% chlorhexidine were used in combination and were efficient in eliminating the bacteria present in and on dog skin.

Many factors limit the bactericidal quality of asepsis techniques, including the type, mode of application, and concentration of the antiseptic used (ARCOS & GOLDMAN, 2010; BUENO, 2010). MCDONALD (2001) suggested that for effective antisepsis, a combination of antiseptics is necessary. According to PEREIRA et al. (1990), increased contact time with chlorhexidine gluconate correlates with a reduction in the number of colony-forming units, resulting in greater residual activity. Therefore, the 100% efficacy achieved using combinations of 2% chlorhexidine surfactant solution + 70% alcohol and 2% chlorhexidine surfactant solution + 0.5% chlorohexidine-alcohol may have been due to the long duration of antisepsis. Further studies are needed to establish whether the protocols used can be equally effective under shorter durations of antisepsis.

CONCLUSION

In conclusion, the combinations of antiseptics tested here, including 2% chlorhexidine surfactant solution + 0.5% chlorhexidine-alcohol and 2% chlorhexidine surfactant solution + 70% alcohol, were effective when administered for 3min. Although, the protocols presented in this study can be safely used for the collection of blood from dogs, removal of hair prior to antisepsis is recommended, because hair tends to accumulate dirt and microorganisms. In addition, dog hair may vary in length, density, and cleanliness, making antiseptics difficult to apply, thereby increasing their failure rate.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This project was previously approved by the appropriate animal ethics committee (CEUA) and was registered under no. 74989.2015.74.

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DECLARATION OF CONFLICT

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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